

FORM PTO-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

BERN-0040

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/913697

INTERNATIONAL APPLICATION NO.
PCT/US00/04427INTERNATIONAL FILING DATE
22 February 2000PRIORITY DATE CLAIMED
22 February 1999

TITLE OF INVENTION

Compositions and Methods for Prevention of Photoaging

APPLICANT(S) FOR DO/EO/US

BERNSTEIN, Eric F.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). - **unexecuted**
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with 37 CFR 1.81.
20. ☐ A second copy of the published international application under 35 U.S.C. 371(c)(2).
21. ☐ A second copy of the English language translation of the international application.
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

- 1) Courtesy copy of International Application
- 2) Copy of Written Opinion
- 3) Return post card

"Express Mail" Label No. **EL886701804US**
Date of Deposit **August 16, 2001**

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

By

Deborah Ehret
Typed Name: Deborah Ehret

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) <div style="font-size: 1.5em; font-weight: bold; margin-top: 5px;">09/913697</div>	INTERNATIONAL APPLICATION NO. <div style="font-weight: bold; margin-top: 5px;">PCT/US00/04427</div>	ATTORNEY'S DOCKET NUMBER <div style="font-weight: bold; margin-top: 5px;">BERN-0040</div>
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531 Rec'd PCT
 16 AUG 2001

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	\$1000.00
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	\$860.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$710.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	\$690.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	\$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$860.00	
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Surcharge of **\$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	10 - 20 =	0	x \$18.00	\$0.00
Independent claims	5 - 3 =	2	x \$80.00	\$160.00
Multiple Dependent Claims (check if applicable). <input type="checkbox"/>				\$0.00
TOTAL OF ABOVE CALCULATIONS =				\$1,020.00

☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.

SUBTOTAL =	\$510.00
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Processing fee of **\$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).

TOTAL NATIONAL FEE =	\$510.00
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Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☐

TOTAL FEES ENCLOSED =	\$510.00
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Applicant, Eric F. Bernstein, is entitled to small entity status. He is an independent inventor.	Amount to be: refunded	\$
	charged	\$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 50-1619 in the amount of \$510.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1619. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Jane Massey Licata, Reg. No. 32,257 Kathleen A. Tyrrell, Reg. No. 38,350 Licata & Tyrrell P.C. 66 E. Main Street Marlton, New Jersey 08053 Telephone: (856) 810-1515 Facsimile: (856) 810-1454	<div style="text-align: center;"> </div> SIGNATURE Jane Massey Licata NAME 32,257 REGISTRATION NUMBER August 16, 2001 DATE
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COMPOSITIONS AND METHODS FOR PREVENTION OF PHOTOAGING

BACKGROUND OF THE INVENTION

The effects of ultraviolet radiation from exposure to the sun on human skin are a growing concern for today's longer-lived population. The majority of changes associated with an aged appearance result from chronic sun-damage (Warren et al., *J. Am. Acad. Dermatol.*, 1991, 25:751-760; Frances, C. and Robert, L., *Int. J. Dermatol.*, 1984, 23:166-179). Dramatic alterations of the superficial dermis accompany the deep wrinkles and laxity common in photoaged skin. The major histopathologic alteration of photoaged skin is the accumulation of material which, on routine histopathologic examination, has the staining characteristics of elastin and is, thus, termed solar elastosis. Immunohistochemical staining has shown the poorly-formed fibers comprising solar elastosis to be composed of elastin (Chen et al., *J. Invest. Dermatol.*, 1986, 87:334-337; Mera et al., *Br. J. Dermatol.*, 1987, 117:21-27) fibrillin (Chen et al., *J. Invest. Dermatol.*, 1986, 87:334-337; Dahlback et al., *J. Invest. Dermatol.*, 1990, 94:284-291; Bernstein et al., *J. Invest. Dermatol.*, 1994, 103:182-186) and versican, the normal components of elastic fibers (Zimmerman et al., *J. Cell. Biol.*, 1994, 124:817-825). A coordinate increase in elastin, fibrillin and versican mRNAs has been demonstrated in fibroblasts derived from photodamaged skin, as compared to fibroblasts derived from normal skin from the same individuals (Bernstein et al., *J. Invest. Dermatol.*, 1994, 103:182-186). Elevated elastin mRNA levels in sun-damaged skin result from enhanced elastin promoter activity, as shown by transient transfections of fibroblasts with a DNA construct composed of the human elastin promoter linked to the chloramphenicol acetyltransferase (CAT) reporter gene (Bernstein et al., *J. Invest. Dermatol.*, 1994, 103:182-186).

Neutrophil elastase has been suggested to be an important mediator in the development of solar elastosis resulting from continued exposure to UVB (See Abstract from Ciba-Found. Symp., 1995, 192:338-46; discussion 346-7). Using an elastase-deficient hairless mouse model and specific small molecular weight elastase inhibitors, it has been shown that attenuation of neutrophil elastase activity results in a pronounced diminuation in the severity of UVB or chemically-induced skin tumors (Starcher et al. *J. Invest. Dermatol.*, 1996, 107:159-163).

A deficiency in alpha 1-antitrypsin has been suggested to allow proteases such as neutrophil elastase to destroy dermal elastin and, thus produce cutis laxa in Marshall's syndrome, a rare pediatric skin disease that is characterized by acquired localized neutrophilic dermatitis (Sweet's disease), followed by loss of elastic tissue in the dermis and cutis laxa (Hwang et al. *Arch. Dermatol.*, 1995, 131(10):1175-7). Alpha 1-proteinase inhibitor, also referred to herein as alpha 1-antitrypsin, is approved by the Food and Drug Administration as a plasma product for the treatment of hereditary alpha 1-antitrypsin deficiency. Alpha 1-antitrypsin has also been disclosed for use in the treatment of atopic dermatitis (Wachter, A.M. and Lezdey, J. *Annals of Allergy*, 1992, 69:407-414).

Alpha 1-antitrypsin is a member of the serine protease inhibitor (serpin) supergene family. Serpins are a superfamily of inhibitors involved in the mediation of a variety of biological processes essential to survival of a host. Members of the serpin family play a role in a great number of biological processes including, but not limited to, inflammation, fertilization, tumor migration, neurotropism, and heat shock. The serpin with the highest naturally occurring plasma concentration is alpha 1-antitrypsin. This serpin has activity toward both tryptic and chymotryptic proteases.

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It has now been found that topical application of serine proteases such as alpha 1-antitrypsin prevents photoaging and other skin damage resulting from exposure to solar, and more specifically, ultraviolet radiation.

5 SUMMARY OF THE INVENTION

In the present invention, a new use is provided for serine proteases such as alpha 1-antitrypsin. It has now been demonstrated that topical application of alpha-1 antitrypsin protects against photoaging and other sun-damage such as
10 sunburn and skin cancer caused by solar radiation. Accordingly, serine proteases with alpha 1-antitrypsin-like activities are believed to be useful as sunscreen agents. Compositions for use as sunscreen agents comprising serine proteases with alpha 1-antitrypsin like activities are also
15 provided.

DETAILED DESCRIPTION OF THE INVENTION

Profound changes take place in the superficial dermis as a result of chronic sun-exposure. The major alteration is the deposition of massive amounts of abnormal elastic material,
20 termed solar elastosis. It has been shown that solar elastosis is accompanied by elevations in elastin and fibrillin mRNAs and elastin promoter activity.

A transgenic mouse model which contains the human elastin promoter linked to a chloramphenicol acetyltransferase (CAT)
25 reporter gene for testing compounds that may inhibit cutaneous photodamage has been developed. These mice express human elastin promoter activity in a tissue-specific and developmentally regulated manner. Promoter activity can be studied in this model as a function of small increases in
30 ultraviolet radiation, demonstrating the sensitivity of the assay. In addition, quantitative data can be obtained after only a single exposure to ultraviolet radiation. A test compound is applied to the skin of a transgenic mouse capable

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of expressing the human elastin promoter. The transgenic mouse is then exposed to solar radiation and human elastin promoter activity in the mouse is determined. The human elastin promoter activity is then compared to that in transgenic mice also exposed to an equivalent dose of solar radiation which were not treated with the test compound to determine whether or not the test compound provided protection against the solar radiation. Since elastin promoter activation is a primary event in cutaneous aging, these mice represent a mouse model of human photoaging.

Using this transgenic mouse line, the ability of alpha 1-antitrypsin to inhibit the effects of solar radiation on human elastin promoter activity was determined. Alpha 1-antitrypsin is produced in the milk of transgenic goats. Accordingly, in these experiments, 5 mice received either no treatment, 10 mice were treated with a 20 mg/ml solution of alpha 1-antitrypsin in goat's milk applied topically to the back, and 10 mice were treated with a solution of goat's milk alone applied topically to the back. A group of mice was also treated with saline only. Approximately fifteen minutes after application of the goat's milk containing alpha 1-antitrypsin, goat's milk alone, or saline these mice were exposed to 20 human minimal erythema doses (MEDs) of solar simulating radiation (SSR). Following phototreatment, the backs of the mice were rinsed twice with 70% isopropyl alcohol pads to remove any excess alpha 1-antitrypsin. This procedure was repeated over three consecutive days.

Mice were sacrificed and skin harvested for determination of CAT activity 24 hours after the third phototreatment. The baseline CAT activity of control mice receiving neither radiation nor alpha 1-antitrypsin was standardized to a value of one. Relative increases in CAT activity were 14.4 ± 3.1 (mean + S.D.) in mice treated with goat's milk alone and 4.5 ± 1.0 in mice treated with goat's milk containing alpha 1-antitrypsin. Thus, topical application of the serpin alpha 1-

antitrypsin produced a 69% reduction in CAT activity. In addition, it was found that milk alone provided 12% protection as compared to the saline control animals.

Accordingly, topical application of a composition comprising alpha 1-antitrypsin or other serpins with alpha 1-antitrypsin like activities to the skin provides protection against photoaging and other sun-damage such as sunburn and skin cancer. By "other serpins with alpha 1-antitrypsin-like activities", it is meant serine protease inhibitors with similar activity toward both tryptic and chymotryptic proteases as alpha 1-antitrypsin. Such serpins include both naturally occurring serine protease inhibitors and mutants rationally engineered to have similar activities and specificity to alpha 1-antitrypsin. Methods of rationally engineering serine proteases and their inhibitors are known. See, for example, Dang et al. *Nature Biotechnology*, 1997, 15:146-149.

Examples of compositions comprising a serpin with alpha 1-antitrypsin like activities include, but are not limited to creams, lotions and sprays. Methods of formulating serpins into creams, lotions and sprays as well as pharmaceutical additives for such formulations are well known to those skilled in the art. As will be obvious to those skilled in the art upon this disclosure, such compositions may further comprise secondary or additional sunscreens or free radical scavengers such as, but not limited to, Vitamin C and Vitamin E and analogs thereof. In a preferred embodiment, a composition comprising a serpin is applied to the skin prior to exposure to the sun. However, application of these compositions subsequent to the exposure can also mitigate any damage resulting to the skin from this exposure. It is believed that these compositions of the present invention will be especially useful in protecting individuals with heightened sensitivities to the sun, such as, but not limited to, individuals undergoing psoralen treatment for cancer, psoriasis and other skin conditions; individuals undergoing photodynamic therapy for

skin cancer, psoriasis and other skin conditions; individuals suffering from genetic repair defects such as xeroderma pigmentosa, albinism or other conditions resulting from decreased endogenous melanin pigment.

5 Further, as demonstrated herein topical application of a composition comprising milk or a product derived therefrom also provides protection against photoaging and other sun-damage such as sunburn and skin cancer. Accordingly, compositions such as creams, lotions and sprays which comprise
10 milk or a product derived therefrom can also be formulated for use in protecting against photodamage and other sun-damage in normal individuals and those with a heightened sensitivity to the sun.

The following nonlimiting examples are provided to
15 further illustrate the present invention.

EXAMPLES

Example 1: Transgenic mice expressing the human elastin promoter

A homozygous line of transgenic mice expressing the 5.2-
20 kb human elastin promoter linked to a CAT reporter gene was used. Hsu-Wong et al., *J. Biol. Chem.*, 1994, 269:18072-18075. These mice express the human elastin promoter in a tissue-specific and developmentally regulated manner. Mice four or five days old were used since at this age, visible hair growth
25 is not yet present.

Example 2: Solar Simulating Radiation

A Multiport Solar Simulator (Solar Light Company, Philadelphia, PA) containing a xenon arc lamp filtered through a Schott WG 320 filter (Schott Glaswerke, Mainz, Germany) was
30 used to administer solar simulating radiation (SSR). The output of the solar simulator was measured by means of a 3D UV meter (Solar Light Company) and displayed as human minimal erythema doses (MEDs). The emission spectrum of the lamp

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closely simulates solar radiation reaching the earth's surface. The light guides from the solar simulator were placed in light contact with the dorsal surface of the mice, which were restrained to prevent movement while SSR was administered.

- 5 Unirradiated control mice were also restrained without receiving SSR.

Example 3: CAT Assay

To measure the expression of the human elastin promoter/CAT reporter gene construct in the skin of transgenic mice and in fibroblast cultures established from these animals, CAT activity was determined. For extraction of the CAT from skin, the specimens were homogenized in 0.25 Tris-HCl, pH 7.5, using a tissue homogenizer (Brinkmann Instruments, Inc. Westbury, NY). The homogenates were centrifuged at 10,000 X g for 15 minutes at 4°C and the protein concentration in the supernatant determined by a commercial protein assay kit (Bio-Rad Laboratories, Richmond, CA). Aliquots of the supernatant containing 100 µg of protein were used for assay of CAT activity by incubation with [¹⁴C] chloramphenicol in accordance with well-known procedures. The acetylated and non-acetylated forms of radioactive chloramphenicol were separated by thin-layer chromatography and CAT activity was determined by the radioactivity in the acetylated forms as a percent of the total radioactivity in each sample.

What is Claimed:

1. A method of protecting humans exposed to sunlight against photoaging, sunburn and skin cancer comprising topically applying to skin of a human a serine protease inhibitor in an amount effective to protect the skin against photoaging, sunburn and skin cancer.
2. The method of claim 1 wherein the serine protease inhibitor is alpha 1-antitrypsin.
3. The method of claim 1 wherein the serine protease inhibitor is applied prior to exposure of the skin to sunlight.
4. The method of claim 1 wherein the serine protease inhibitor is applied subsequent to exposure of the skin to sunlight.
5. A method of protecting individuals with a heightened sensitivity to the sun from damage resulting from the sun comprising topically applying to the skin of an individuals with a heightened sensitivity to the sun a serine protease inhibitor prior to exposure of the individual to the sun.
6. The method of claim 5 wherein the serine protease inhibitor is alpha 1-antitrypsin.
7. A method of protecting humans exposed to sunlight against photoaging, sunburn and skin cancer comprising topically applying to skin of a human milk or a product derived from milk.
8. A pharmaceutical composition for prevention of photoaging and other sun-damage comprising a serine protease inhibitor, a second sunscreen or free radical scavenger, and a pharmaceutical additive.

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9. The pharmaceutical composition of claim 7 wherein the serine protease inhibitor is alpha 1-antitrypsin.

10. A pharmaceutical composition for prevention of photoaging and other sun-damage comprising milk or a product
5 derived therefrom and a pharmaceutical additive.

ACCEPTED

Docket No.
BERN-0040

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Compositions and Methods for Prevention of Photoaging

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 22 February 2000 as United States Application No. or PCT International Application Number PCT/US00/04427 and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>

I hereby claim the 'benefit' under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/121,118

February 22, 1999

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

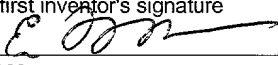


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2003-01-26 09:55:50

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